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Published in:
Journal of Clinical Endocrinology & Metabolism

DOI:
10.1210/jc.2009-2589

JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

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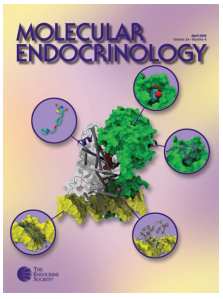
J. Clin. Endocrinol. Metab. 2010 95:2851-2855 originally published online Apr 9, 2010; , doi: 10.1210/jc.2009-2589



Elevated Urinary Free and Deconjugated Catecholamines after Consumption of a Catecholamine-Rich Diet

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Context: The biochemical diagnosis of pheochromocytoma depends on the demonstration of elevated levels of catecholamines (*i.e.* epinephrine, norepinephrine, and dopamine) and their metabolites.

Objective: The aim of the study was to determine the preanalytical influence of a catecholamine-rich diet on urinary free and deconjugated catecholamines in healthy volunteers with a highly specific and sensitive analytical technique.

Design, Setting, and Participants: We conducted a crossover study involving 27 healthy adults in a specialist medical center.

Interventions: Subjects consumed catecholamine-rich nuts and fruits at fixed times on one day (about 35 μmol dopamine and 1 μmol norepinephrine) and catecholamine-poor products on another day. Urine samples were collected at timed intervals before, during, and after experimental and control interventions.

Main Outcome Measures: We performed automated online sample preparation coupled to isotope-dilution mass spectrometry measurements of urinary concentrations of free and deconjugated catecholamines.

Results: The catecholamine-rich diet had substantial effects on urinary excretions of deconjugated dopamine (up to 20-fold increases) and norepinephrine (up to 10-fold). Dietary catecholamines had less but significant effects on urinary excretion of free dopamine and norepinephrine (up to 1.5-fold increases). Outputs of urinary free and deconjugated epinephrine remained unaffected.

Conclusions: Urinary excretion of deconjugated norepinephrine and dopamine is strongly affected by consumption of catecholamine-rich food products, thereby increasing the likelihood of a false-positive test result during hormonal evaluation for pheochromocytoma. Measurement of deconjugated catecholamines should therefore preferably be avoided, in favor of measurement of urinary free catecholamines. In case of demonstrating increased urinary excretion of deconjugated norepinephrine and dopamine, repeated measurements are warranted with dietary restrictions prior to sample collection. **(*J Clin Endocrinol Metab* 95: 2851–2855, 2010)**

The biochemical diagnosis of pheochromocytoma is based on demonstration of elevated concentrations of compounds in the catecholamine metabolic pathway, *i.e.* urinary and plasma fractionated catecholamines (norepinephrine, epinephrine, dopamine) and their *O*-methylated metabolites (metanephrines) (1, 2). Several authors recommend measurements of plasma metanephrines for the diagnosis of pheochromocytoma (3–5), whereas others advocate the plasma test in combination with urinary measurements of catecholamines (6, 7). Currently, quantitative assays applied are state of the art, well-performing, and insensitive to analytical interference. However, biochemical results may be affected by preanalytical factors,

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-2589 Received December 7, 2009. Accepted March 15, 2010.
First Published Online April 9, 2010

such as physiological influences (*e.g.* exercise, posture, stress) and medications (*e.g.* catecholamine reuptake blockers) that alter the production or disposition of catecholamines. We have demonstrated before that consumption of foods containing substantial quantities of biogenic amines [*i.e.* fruits and nuts (8)] increase levels of urinary and plasma metanephrines (9).

The main fraction of catecholamines is converted to sulfate conjugates by a sulfotransferase isoenzyme (SULT1A3), located in the gastrointestinal tract, that inactivates both endogenous and dietary-derived (exogenous) catecholamines (10, 11). Therefore, catecholamine outputs can be presented as free or deconjugated (sum of free and sulfate-conjugated) concentrations. The aim of this study was to examine the potential preanalytical influence of a catecholamine-rich diet on urinary free and sulfate-conjugated catecholamines in healthy volunteers. Previous studies on this subject (12) were unable to distinguish between analytical interferences and physiological influences. Therefore, by application of an analytical state of the art automated isotope-dilution mass spectrometric method, this study has for the first time been performed interference-free.

Subjects and Methods

Analytical

Automated online sample preparation coupled to isotope-dilution liquid chromatography-mass spectrometry was applied for the measurement of urinary concentrations of free and deconjugated epinephrine, norepinephrine, and dopamine. Immediately after collection, urine samples were acidified to pH 4, and preservatives ascorbic acid and EDTA were added. Samples were stored at −20 C. Urine samples were diluted with deuterated internal standard and ascorbic acid solution (400 mg/liter) and injected into the analysis system.

Diet experiment

Subjects included 27 healthy adults (14 women, 13 men; median age, 38 yr; range, 21–59 yr) who served as their own controls by participating in both control and experimental arms of the protocol following a crossover design, with at least 1 wk between the randomly distributed test days. Both arms were preceded by an overnight fast from at least 2400 h until 0830 h. Subjects avoided catecholamine-containing products (*e.g.* fruits, fruit drinks, nuts, potatoes, tomatoes, and beans) the day before, during, and the morning after both study days, as described previously (9).

For the experimental arm, all subjects consumed two catecholamine-rich meals. The first meal, consumed at 0830 h, included two or three bananas (280 g of pulp), one quarter of a fresh pineapple (185 g of pulp), 50 g of shelled walnuts, and 140 ml of pineapple juice purchased at local commercial outlets. At 1030 h, each subject drank 280 ml of pineapple juice. At 1230 h, they consumed a second meal similar to the first. Finally, at 1430 h, participants drank 280 ml of pineapple juice. Based on previous measurements (13), total dopamine and norepinephrine intakes were estimated at 35 and 1 μmol, respectively; epinephrine was undetectable.

For the control arm, the subjects consumed meals (bread), snacks (gingerbread), and drinks (coffee, tea, dairy products) in accordance with the time schedule of the experimental arm. Morning urine until 0830 h was gathered from all subjects. Four separate urine samples were then collected at 2-h intervals starting at 1030 h, with the fourth collection at 1630 h. A final urine specimen was collected beginning at 1630 h and ending at 0800 h the next morning. Aliquots were stored within 1 d at −20 C until analysis. The study was approved by the medical ethics committee of our institution and conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent. The study has been registered at the following Dutch on-line register: https://toetsingonline.ccmo.nl/ccmo_search.nsf/Searchform?OpenForm.

Data analysis

Results from the influence of diet on the free and total catecholamine levels are shown as mean values with 95% confidence intervals. First morning urine samples (until 0830 h) served as reference points for dietary-associated changes over time. Linear mixed models, tested for significance at $P < 0.05$, were used to determine the significance of temporal changes in analyte concentrations (14). The model fit was evaluated for deviance and performed using the statistic software program ML Win version 2.0.2 (Centre for Multilevel Modeling, Bristol, UK). Time was included as a factor (fixed and random). The magnitude of a difference between the control and the experimental groups in this modeling is given by the interaction term between diet and time because no differences at baseline concentrations between both groups were expected.

Results

Urinary dopamine

Outputs of free and deconjugated dopamine were higher after ingestion of the high-catecholamine meals compared with control meals ($P < 0.05$). After consumption of the second catecholamine-rich meal, outputs of free dopamine (Fig. 1A) were 1.5-fold ($P < 0.05$) and deconjugated dopamine more than 20-fold higher ($P < 0.05$) than baseline values (Fig. 1B). Free dopamine returned to baseline values overnight, whereas deconjugated dopamine remained elevated but decreased significantly from values of the preceding collection. The upper reference limit (206 μmol/mol creatinine) was exceeded by two subjects between 1030 and 1430 h, by nine subjects between 1230 and 1430 h, and by four subjects between 1430 and 1630 h. No significant changes in concentrations of free or deconjugated dopamine were apparent after ingestion of the control meals.

Urinary norepinephrine

Free norepinephrine showed daytime increases and nighttime decreases ($P < 0.05$) during the 24-h period

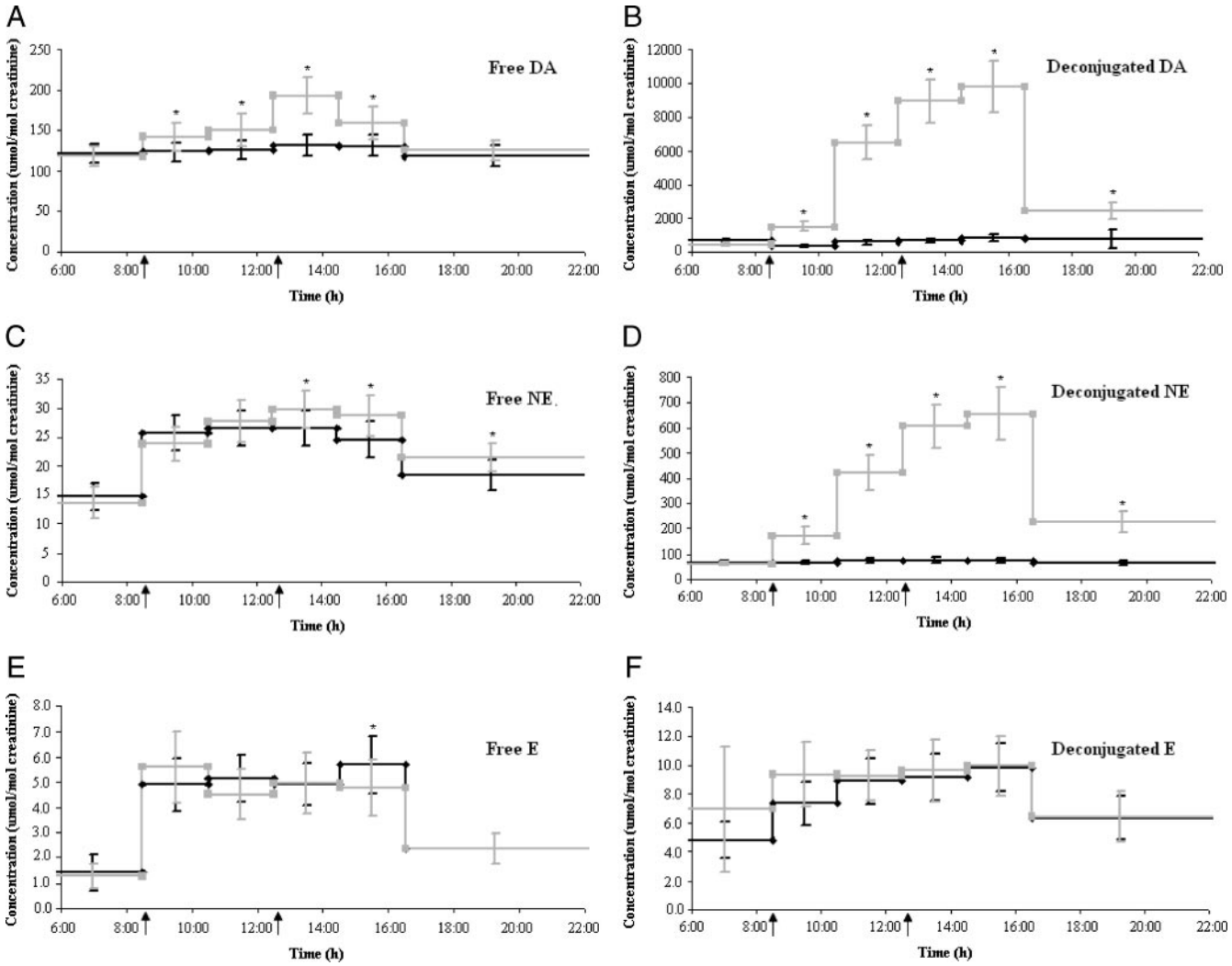

FIG. 1. Line graphs of mean urinary concentrations of catecholamines before, during, and after catecholamine-rich and catecholamine-poor meals. Means were calculated from data of 27 subjects in both the control and the diet group and expressed with the 95% confidence intervals. Obtained statistic linear mixed models showed comparable graphs. Therefore, changes in concentrations in these models are significant ($P <$ 0.05). *Asterisks* indicate significant differences between control and diet group data ($P < 0.05$). A, Urinary free dopamine (upper reference limit, 206 μmol/mol creatinine); B, urinary deconjugated (free + sulfate-conjugated) dopamine; C, urinary free norepinephrine (upper reference limit, 43 μmol/mol creatinine); D, urinary deconjugated norepinephrine; E, urinary free epinephrine (upper reference limit, 8 μmol/mol creatinine); and F, urinary deconjugated epinephrine. *Arrows* indicate the times at which test meals were taken (0830 and 1230 h). *Gray line*, Significant model for the diet group (n = 27); *black line*, significant model for the control group (n = 27). E, Epinephrine; NE, norepinephrine; DA, dopamine.

after both catecholamine-rich and control meals (Fig. 1, C and D). Free norepinephrine excretion increased significantly more after the second meal in the diet group than in the controls and remained elevated overnight. The upper reference limit (43 μmol/mol creatinine) was exceeded by two subjects between 1030 and 1430 h, two subjects between 1230 and 1430 h, and by three subjects between 1430 and 1630 h. In addition, deconjugated norepinephrine showed significant ($P < 0.05$) increases after ingestion of control *vs.* experimental meals up until the following morning (Fig. 1D). On the day of the catecholamine-rich diet, deconjugated norepinephrine increased by 10-fold over baseline values for the collection between 1430 and 1630 h and remained nearly 4-fold higher for the overnight collection.

Urinary epinephrine

Free and deconjugated epinephrine (Fig. 1, E and F) showed the same time course of changes after control and experimental meals, indicating no influence of the catecholamine-rich diet. Deconjugated epinephrine, and to a lesser extent urinary free epinephrine, showed significantly higher ($P < 0.05$) excretions during daytime compared with nighttime.

Discussion

The current study, performed with an analytical state of the art automated isotope-dilution mass spectrometric method, shows that diet composition substantially influ-

ences sulfate-conjugates of catecholamines present in urine. These findings are in agreement with our recent study on dietary influences of urinary and plasma metanephrines (9). However, ingestion of catecholamine-rich foods has a more profound effect on urinary excretion of sulfate-conjugated catecholamines (up to 20-fold elevation) than on that of sulfate-conjugated metanephrines (up to 3-fold elevation) (9). This is explained by the fact that ingested catecholamines are mainly converted to sulfate conjugates in the gastrointestinal tract by SULT1A3 (10, 11), whereas only a small fraction is metabolized to metanephrines. Consequently, consumption of catecholamine-containing foods may significantly increase urinary excretion of sulfate-conjugated catecholamines (10, 11), indicating that diet restrictions before sampling should be established. In general, measurement of urinary deconjugated catecholamines has already been replaced by the measurement of urinary free catecholamines. In the present study, however, it is demonstrated that the free fractions of norepinephrine and dopamine are also significantly affected by diet composition, albeit to a substantially lesser extent than the deconjugated fractions. Therefore, diet restriction should also be considered preceding measurement of urinary free catecholamines. In addition, attention should be paid to the addition of acid preservatives against oxidation of catecholamines. Sulfate-conjugated catecholamines are sensitive to hydrolysis in an acid environment, resulting in increased levels of the free fraction (15). This will be especially important when conjugated levels are relatively more elevated than free levels due to dietary composition.

Mean results for free catecholamine levels of 26 subjects did not result in a rise of catecholamine levels above the upper reference limits (limits for deconjugated catecholamines are unknown), although some individuals slightly crossed those limits after both meals. Of notice, the total amounts of catecholamines consumed in the present study were not extraordinarily high because regular Western meals may contain more than 10 times the amount of catecholamines (16). Therefore, our findings probably undervalue the occurrence of false-positive test results after ingestion of catecholamine-rich foods. In addition, catecholamines are present in a large variety of foods [other than fruits and nuts (8)] in amounts that have not been precisely delineated, such as tomatoes, beans, and cheeses. Besides, dietary influences may not be confined to food products that contain catecholamines, as we discussed previously (9). Bananas have been demonstrated to contain the highest amounts of dopamine of all known catecholamine-containing foods and are thus quantitatively the most important dietary product to be considered (13, 17).

Our results are in agreement with some (12, 17), but not all (18), previous studies describing the influence of diet on catecholamine excretion. Discrepancies are likely to be explained by differences in assay technique and test protocols. Previous studies were performed at least two decades ago and did not use the state-of-the-art equipment of the current study. In one study, the effect of a normal diet was examined, but not the effect of a catecholamine-rich diet (19).

The present study also shows daytime increases and nighttime decreases in circulatory outputs of urinary free and deconjugated catecholamines, consistent with previous observations (20) likely reflecting increased sympathoadrenal outflow related to a more ambulatory and active status during waking hours. This biological variation emphasizes the need to collect 24-h urine samples instead of urine portions. Concurrently, effects of diet on the excretion of free catecholamines will be diminished due to dilution of the urine over the 24-h collection period.

In conclusion, urinary excretion of deconjugated catecholamines and, to a lesser extent, of free catecholamines, is affected by consumption of catecholamine-rich food products, thereby increasing the likelihood of false-positive biochemical test results for the diagnosis of pheochromocytoma. We therefore recommend repeated measurement of urinary free catecholamines to confirm the presence of an increased urinary catecholamine excretion. Dietary restrictions 24 h before this repeated sampling should be sufficient to circumvent increased levels by consumption of catecholamine-containing foods because this study showed that overnight catecholamine levels are returning to normal. Alternatively, one could measure plasma free metanephrines, which are not affected by dietary catecholamines (9). Thus, the results of our study further strengthen the recommendation of plasma free metanephrines as the biochemical test of choice for the diagnosis of pheochromocytoma (3).

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

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